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Grade of chemotherapy-induced necrosis as a predictor of local and systemic control in 881 patients with non-metastatic osteosarcoma of the extremities treated with neoadjuvant chemotherapy in a single institution

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Abstract

To determine whether necrosis induced by pre-operative chemotherapy correlates with the rate of systemic and local relapse, may change the pattern of relapse and/or may modify the chance of success of post-relapse treatments, we evaluated 881 patients with non-metastatic osteosarcoma of the extremities treated with five different protocols of neoadjuvant chemotherapy and surgery at the same institution between 1983 and 1999. The 5-year disease-free survival (DFS) and overall survival (OS) correlated significantly with the histological response to chemotherapy. Five-year DFS and OS in good and poor responders were 67.9% *versus* 51.3% ($P < 0.0001$) and 78.4% *versus* 63.7% ($P < 0.0001$), respectively. The prognostic value of the histological response was valid only for osteoblastic and telangiectatic osteosarcoma subtypes. Nonetheless, since they represent more than 70% of all osteosarcomas, we conclude that chemotherapy-induced necrosis has a significant prognostic value, regardless of the type of chemotherapy performed after surgery.

Keywords: Osteosarcoma; Chemotherapy; Necrosis; Prognosis

1. Introduction

Induced pathological necrosis has been shown to be the best prognostic factor for patients with osteosarcoma of the extremities [1–10]. However, in order to grade the histological response to chemotherapy, different criteria of evaluation have been used. Huvos and colleagues [11] proposed a 4-grade scale, Salzer-Kuntschik and colleagues [12] a 6-grade scale, Ayala and colleagues [13] a 3-grade scale. Many others authors [1–3,9,14,15] expressed these data as percentages of necrotic tissue, thus good responder patients were those with necrosis $\geqslant 95\%$ [1,4–6], $\geqslant 90\%$ [6,7,14,15] or $\geqslant 60\%$ [2,3,9]. For these reasons, a comparison between different series is impossible and, up to now, there have not been large series of patients from a single institution to verify the real prognostic value of chemotherapy-induced necrosis. Moreover, there are no studies analysing differences in patterns of relapse and outcome of post-relapse treatment according to histological response to pre-operative chemotherapy.

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The aim of this study was to determine the prognostic significance of histological response to pre-operative treatment in 881 patients with non-metastatic osteosarcoma of the extremities treated at the same institution, as well as to evaluate whether the pattern of relapse and post-relapse outcome are different in good and poor responder patients.

2. Materials and methods

2.1. Patient selection, pathology and pre-operative evaluation

Patients were considered eligible for the neoadjuvant trials if they fulfilled the following criteria: typical radiographical and histological features of primary, high-grade, central osteosarcoma; tumour located in the extremity; no previous history of cancer and no prior treatments; age under 40 years; no associated disease contraindicating chemotherapy; and no evidence of metastases at diagnosis. Of the newly diagnosed cases of osteosarcoma observed at the authors' institution between March 1983 and June 1999, 900 patients were eligible for the study. All the eligible patients were offered a neoadjuvant treatment after having been informed of the potential advantages and risks. Of the 900 eligible patients, 19 declined to enter the study so they were treated at our institution: only by surgery (10), by adjuvant chemotherapy (5), or moved to other centers (4). The remaining 881 patients, entered the study. Their characteristics are shown in Table 1.

The diagnosis of osteosarcoma, established by clinical and radiological findings, was always confirmed on histological slides of tumour tissue obtained from an open or needle biopsy as well as from the resected specimen. Osteosarcomas were classified as 'classic', telangiectatic, small cell osteosarcoma. On the basis of predominant cells and intercellular material, 'classic' osteosarcoma were classified into the following subtypes: osteoblastic, fibroblastic and chondroblastic. This distinction, always made on surgical specimens, was possible in all but 78 cases, which were defined as 'classic-non-classifiable'. All patients had a complete history taken, a thorough physical examination and several biochemical tests. The primary tumour was evaluated on standard radiographs, technetium-99 methylenediphosphonate (Tc-99 MDP) bone scans, and computed tomography (CT) scans. Magnetic resonance imaging (MRI) was performed only in the 220 most recent cases. Bone metastases were investigated with total body scan, whereas CT scans of the chest were used to exclude lung metastases.

Table 1
Histological response according to patients' characteristics

Characteristic	Number of cases (total = 881)	Good responders (%)	Poor responders (%)	*P*
Age (years)				
⩽14	367	62	38	0.17
>14	514	60	40	
Gender				
Male	511	59	41	0.07
Female	370	65	35	
Volume				
⩽150 ml	389	51	35	0.08
>150 ml	419	49	65	
Pathological fracture	62	8	6	0.23
Site				
Femur	465	63	37	0.29
Tibia	240	62	38	
Humerus	109	57	43	
Other bones	67	54	46	
Serum alkaline phosphatase				
Normal	547	63	37	0.31
Elevated	334	59	41	
Histological subtype				
Osteoblastic	574	62	38	0.0001
Chondroblastic	85	51	49	
Fibroblastic	76	60	40	
Telangiectatic	62	87	13	
Not classifiable	78	52	48	
Others	6	67	33	

2.2. Chemotherapy, surgery, pathological evaluation and follow-up

Patients were treated according to five different neoadjuvant protocols of chemotherapy, with different, pre- and post-operative combinations of methotrexate, cisplatinum, doxorubicin and ifosfamide, successively activated, as summarized in Table 2, and reported in detail previously [14,18–21].

Surgery was scheduled 3 weeks after the end of pre-operative chemotherapy. Before surgery, the tumour was re-staged to assess its extension after pre-operative treatment. The type of surgery (amputation, rotation plasty or limb salvage), as well as the type of reconstruction after resection (prosthesis, Kunstscher rod or plate and cement, vascularized fibula combined with allograft, allograft and autograft) were chosen depending on the location and extension of the tumour, neurovascular structure involvement, skeletal maturity, desired lifestyle and presence of complicating factors, such as displaced pathological fractures or infected biopsy sites. In order to perform conservative surgery, it was always mandatory that the pre-operative staging assured the possibility of achieving wide surgical margins together with limb partial functionality.

After surgery, surgeons and pathologists reviewed the gross specimens to determine surgical margins, that were classified 'adequate' if radical or wide, and 'inadequate' if marginal, intralesional, or contaminated, according to Enneking's classification [16]. The histological response to chemotherapy was evaluated following the method previously reported [17] and the grade of necrosis expressed as a percentage of dead tumour cells. The histological response was graded as 'good' (90% or more tumour necrosis) or 'poor' (less than 90% tumour necrosis). These two grades correspond approximately to grades III and IV and to grades I and II of the descriptive classification proposed by Rosen and colleagues [1] and followed by many authors.

During post-operative chemotherapy, besides the clinical evaluation, patients were checked every 2 months with radiographs or CT scan of the chest and the operated limb, depending on the date of the control. Additional investigations were performed only if there was a clinical and/or radiographic suspicion of relapse. After completion of chemotherapy, patients were followed in the outpatient clinic with radiographs and/or CT scans every 2 months for 2 years, every 3 months in the third year and then every 6 months.

2.3. Post-relapse treatment

The type of treatment performed to treat metastases and local recurrence in relapsed patients was not standardized but performed on an individual basis, depending on the type of relapse, local and/or systemic, the site and number of metastases, the length of the relapse-free interval and the type of chemotherapy previously received. In addition, since our study considered patients treated over a very long period, methods for diagnosis and surgical techniques were different, so the time of relapse determined the type of treatment received. Nonetheless, the key point of treatment for all patients followed at our institution was the surgical removal of metastases and/or of local relapse whenever possible. A second-line chemotherapy, performed with drugs not previously used or with higher doses of pre-relapse treatment drugs, was generally given, with the exception of those patients who relapsed with only one or two pulmonary metastases after a relapse-free interval of 2 or more years. When, after the first relapse, further relapses occurred, no codified treatments were performed. It must also be remembered that most patients preferred to move to other institutions after the second relapse.

2.4. Statistics

Since the major aim of the study was to correlate disease-free survival (DFS) to the histological response to chemotherapy, 13 patients who died of chemotherapy toxicity and six who died of causes unrelated to tumour or treatment were not considered. Only metastases and

Table 2
Protocols of neoadjuvant chemotherapy

Protocol (references)	Period	Pre-operative treatment	Post-operative treatment
IOR/OS-N1 [14]	1983–1986	HDMTX–CDP *versus* MTX–CDP	Good responders: MTX–CDP ADM Poor responders: ADM–BCD
IOR/OS-N2 [21]	1986–1989	MTX–CDP–ADM	Good responders: MTX–CDP–ADM Poor responders: MTX–CDP–ADM–IFO–ETO
IOR/OS-N3 [19]	1990–1993	MTX–CDP–LDADM	Good responders: MTX–CDP–LDADM Poor responders: MTX–CDP–LDADM–IFO–ETO
IOR/OS-N4 [18]	1994–1995	HDMTX–CDP–ADM–IFO	Good & poor responders: HDMTX–CDP–ADM–IFO
IOR/OS-N5 [20]	1996–1999	HDMTX–CDP–ADM–HDIFO	Good responders: HDMTX–CDP–ADM–HDIFO (3 cycles) Poor responders: HDMTX–CDP–ADM–HDIFO (4 cycles)

MTX, methotrexate (LD, low-dose, HD, high-dose); CDP, cisplatin; ADM, doxorubicin (LD, low-dose, HD, high-dose); BCD, bleomycin + cyclophosphamide + dactinomycin; IFO, ifosfamide (LD, low-dose, HD, high-dose); ETO, etoposide.

local recurrences were considered adverse events in this study. Overall survival (OS) was also evaluated, but the relative data should be considered with caution. When recurrent disease occurred, the post-relapse treatment was not homogeneous and changed markedly during the 16-year study period.

DFS was calculated from the first day of pre-operative chemotherapy to the first adverse event or to the most recent follow-up examination; OS was calculated from the first day of chemotherapy until death. The survival curves were calculated according to the Kaplan–Meier method and compared by means of the long-rank test. Cox proportional hazards regression analyses was used for multivariate analyses, to test predictive factors for survival. The frequency of distribution of different parameters was compared among groups of patients by means of the χ^2 test. Significance was set at $P < 0.05$.

3. Results

3.1. Surgery

As regards surgery, 753 patients (85.4%) were treated with limb salvage, 90 (10.2%) with amputation and 38 (4.2%) with rotation plasty. According to the histological response to pre-operative surgery the rate of limb salvage was significantly higher for good responder patients than for poor responders (89.5% *versus* 79.3%, $P < 0.0001$). The surgical margins were adequate in 811 patients (92%) and inadequate in 70 (8%). According to the histological response to chemotherapy the surgical margins were adequate in 91.8% of good responders and 92.3% in poor responders ($P < 0.91$).

3.2. Histological response to pre-operative treatment

As reported in Table 1, for patients who received a neoadjuvant treatment, the chemotherapy-related tumour necrosis was good in 542 (62%) and poor in 339 (38%). The rate of good histological responses was not related to patient's age, site or size of tumour, or to serum levels of alkaline phosphatase (AP) at presentation. The rate of good histological response was slightly better for females than for males ($P < 0.07$) and in patients with smaller tumours ($P < 0.08$). However neither differences were significant. According to the histological subtype, patients with chondroblastic tumours showed a significantly lower percentage of good responses compared with the other subtypes (50% *versus* 63%, $P < 0.01$), while the telangiectatic osteosarcoma had a significant higher rate of good histological response (87% *versus* 60%, $P < 0.0001$). According to the chemotherapy protocol, the rate of good histological response was significantly lower for the 127 patients who pre-operatively received a two-drug regimen than the rate of the 370 and 384 patients who received a three- or four-drug regimens, respectively (46%, 59%, 64%; $P < 0.0005$). Among patients pre-operatively treated with a three- or four-drug regimen there were no significant differences in terms of histological response.

3.3. Event-free survival

At a median follow-up of 11 years, 531 patients (59%) remained continuously event-free, 350 relapsed (38.9%), 12 died of chemotherapy toxicity (1.3%) and 7 died of other reasons (two suicides, two pulmonary embolisms, 1 complication of central venous catheter (CVC), 1 car crash and 1 second malignancy). The 5-year event-free survival (EFS) and OS for all the 900 eligible patients were 60.2% (95% CI 51–68%) and 71.2% (95% CI 54–88%), respectively, while if we consider only the 881 patients who entered the study and were evaluable for histological response as a prognostic factor, DFS and OS are 61.5% (95% CI 45–77%) and 72.7% (95% CI 55–88%), respectively.

At the univariate analysis, the 5-year DFS rate was not related to gender, site and volume of the tumour, presence of pathological fracture or surgical margins; while it was significantly higher in patients with normal values of AP: 71.2% (95% CI 63–79%) *versus* 45.9% (95% CI 28–63%) ($P < 0.0001$) in patients surgically treated with limb salvage or rotation plasty: 63.1%, 59.5%, 48.9% ($P < 0.03$) and for poor responders to pre-operative chemotherapy: 68.1% (95% CI 50–86%) *versus* 31.9% (95% CI 209–56%) ($P < 0.00001$). At the multivariate analyses (Table 3), the type of surgery lost its prognostic significance. It is important to stress that the prognostic value of the histological response was true for all but one of the five protocols. In fact, chemotherapy-induced necrosis was not a significant prognostic factor in protocol IOR/OS-N5. Among the 410 good responders, we further compared those with necrosis between 90% and 99% and patients with total necrosis, and the rate of 5-year DFS were 65.6% (95% CI 55–76%) *versus* 75.0% (95% CI 58–90%), respectively. This difference is not significant ($P < 0.05$). In addition, among the 339 poor responder patients we made a comparison between

Table 3
Multivariate analysis for prognostic factors

	Relative risk	95% CI	Wald test
Serum alkaline phosphatase			
Normal	1	1.71–2.76	$P < 0.0005$
Elevated	2.18		
Histological response			
Good	1	1.53– 2.47	$P < 0.0005$
Poor	1.94		

CI, confidence interval.

those 200 who received a salvage post-operative chemotherapy with drugs different from pre-operative ones, and those 139 who had a post-operative treatment equal to that used before surgery, and we found rates of DFS and OS surprisingly higher for the second group: DFS = 46.0% (95% CI 29–65%) *versus* 58.9% (95% CI 51–66%), $P < 0.024$; OS = 58% (95% CI 52–75%) *versus* 71% (95% CI 64–79%), $P < 0.022$.

Evaluating the 5-year DFS according to the histological response to chemotherapy and different osteosarcoma subtypes (Table 4), good responders with osteoblastic and telangiectatic tumour variants had a significantly better prognosis than those with fibroblastic, chondroblastic and no classifiable tumours.

3.4. Local recurrence

Local relapses occurred in 50 patients (5.7%), 51–20 months (median 25.9 months) from the beginning of treatment. In all but two cases local recurrence was combined with systemic relapse. In 19 patients local recurrence occurred from 3 to 28 months (median = 8 months) before metastases, in 13 metastases were diagnosed 4–32 months (median = 11 months) before local recurrences and in 18 cases local and systemic relapses were contemporary. The rate of local recurrence was 3.3% for amputated patients, 5.4% for the rotation plasties and 5.9% for patients treated with limb salvages. These differences are not statistically significant. The rate of local recurrence significantly correlated with the surgical margins: local recurrences were 3.6% for the 811 patients with radical or wide margins and 24% for the 70 patients with marginal or intralesional margins ($P < 0.0001$). No significant differences were found according to the histological response to chemotherapy, in fact the rate was 7.4% (25/339) in poor responders and 4.6% (25/542) in good responders ($P < 0.11$). Seven local recurrences were observed in the 132 patients with total necrosis.

3.5. Pattern of relapse

The 350 patients who relapsed did so for local recurrence in two cases (0.6%), for local recurrence plus metastases in 48 (13.7%) and with metastases alone in 300 (85.7%). As reported in Table 5, the rate of local relapses was the same for good and poor responder patients (4.6% *versus* 7.4%, $P < 0.08$). On the contrary, the rate of systemic relapse without local recurrences was significantly higher in poor responders (50% *versus* 29%, $P < 0.0001$). The first site of metastases was the lung in 305 patients (87.1%), other bones in 33 (9.4%), contemporary in bone and lung in two patients and other sites in eight. There were no differences in term of site of first relapse between patients who were good and poor responders: the rate of first metastases in the lung was 87.8% and 86.4%, respectively. The average time to relapse was 21.3 months (range 2–204), and it was significantly longer in good responders in comparison with poor responders (25.7 months *versus* 21.4 months, $P < 0.03$). In addition, the time to death in patients who died was significantly longer in good responder than in poor responders (41.4 months *versus* 32.3 months, $P < 0.004$).

Table 4
Five-year disease-free survival according to histological response to chemotherapy of different osteosarcoma subtypes

Osteosarcoma subtype	Number of cases (total = 881)	DFS in good responders (%)	DFS in poor responders (%)	*P*
Osteoblastic	574	63	46	0.002
Chondroblastic	85	64	56	NS
Fibroblastic	76	83	70	NS
Telangiectatic	62	81	37	0.02
Not classifiable	78	78	70	NS
Others	6	50	50	NS

NS, not significant.

Table 5
Results according to histological response

	Good responders (542 patients)	Poor responders (339 patients)	*P*
5-Year disease-free survival (DFS)	68%	51%	0.0001
5-Year overall survival (OS)	78%	63%	0.0001
Systemic relapse	29%	50%	0.0001
Local recurrence	4.6%	7.4%	0.08
Mean time to relapse (months)	26	21	0.03
Patients alive and free of disease after relapse	26%	22%	0.4
Mean time to death (months)	41	32	0.004

3.6. Post-relapse outcome

After the first relapse, 25 patients moved to other institutions for treatment and seven were lost to follow-up. For the 318 cases treated and followed at our institution the number of patients currently alive and free of disease after 14–210 months from relapse was: 26.8% for poor responders and 26.1% for good responders. The 5-year DFS after relapse in these two groups were 20.2% (95% CI 9–30%) and 19.8% (95% CI 2–39%). This difference is not significant.

4. Discussion

Treatment-induced pathological necrosis has been proven a predictor of survival in patients who receive neoadjuvant chemotherapy for osteosarcoma of the extremities [1–10]. This study, performed on a large number of patients treated in a single institution, whose histological response to pre-operative chemotherapy was evaluated by the same pathologists, confirmed this result. At a multivariate evaluation, the outcome of patients was significantly related only to the grade of histological response to chemotherapy and to the serum level of AP. The rates of 5-year DFS and OS were significantly higher in good responders than in poor responders. This relationship between histological response to pre-operative treatment and long-term outcome suggested that the effect of chemotherapy on the primary tumour correlated with its effect on microscopic disease. Of the 881 patients evaluated, there were 542 (62%) good responders and 339 (38%) poor responders. The rate of good histological response correlated significantly with the number of drugs used before surgery and with the histological subtype of the tumour. Our evaluation was made given the data for necrosis as percentage of dead cells with a cut-off level of 90% of necrosis. A further division of our good responders into patients with total necrosis *versus* those with necrosis between 90% and 99% showed no significant differences. It is important to stress that the prognostic significance of necrosis was valid for all but one of the five different protocols used, with no differences between protocols with a post-operative salvage chemotherapy and protocols with a post-operative treatment equal to that performed before surgery. Thus, based on our experience, the modification of chemotherapy after surgery, first suggested by Rosen and colleagues [1] and adopted by many others [3–5,7] does not seem to provide any advantage. Many other authors have also reported this same experience [5,15,17]. Nonetheless, the prognostic significance of histological response to chemotherapy was not valid for all osteosarcoma subtypes. In fact, in chondroblastic and fibroblastic tumours the rate of DFS was almost the same for good and poor responders. As a consequence, the rate of good responder, chondroblastic patients is lower than that of osteoblastic patients, as also reported previously by others [12], but their final prognosis is the same. We observed a longer relapse-free interval for good responder patients, but, in spite of later relapses, the good responders do not have a significantly higher probability of long-term survival after the salvage treatment in comparison with poor responders. Our study also showed a significantly higher rate of limb salvages in good responders (83% *versus* 93%, $P < 0.0001$). However it is important to remember that the current study considered patients who were treated over long period of time, during which the approach to osteosarcoma patients underwent some changes. For instance, the higher rate of poor responders was observed in patients treated with the first protocol between 1983 and 1986. At that time MRI and some reconstructive techniques were not available for all patients. More recently, new diagnostic techniques, new tools for reconstruction, and increased experience and confidence with limb-salvage procedures might have further benefited good responder patients.

As regards local recurrence, the rate was almost the same in good and poor responders. That seems to indicate that the increased rate of DFS in good responders is due only to the lower number of systemic relapses. On the basis of our results, we conclude that in the neoadjuvant treatment of osteosarcoma all the effective drugs must be employed pre-operatively at the highest dose, in order to achieve the highest rate of good histological response. Addition of other drugs or changes to post-operative chemotherapy regimens in poor responder patients does not improve their outcome. Moreover, surgeons have to be fully aware that the risk of local recurrence is strictly connected with surgical margins but not with histological response.

Conflict of interest statement

None declared.

References

1. Rosen G, Caparros B, Huvos AG, *et al*. Preoperative chemotherapy for osteogenic sarcoma: selection of postoperative adjuvant chemotherapy based on response of the primary tumor to preoperative chemotherapy. *Cancer* 1982, **49**, 1221–1230.
2. Winkler K, Beron G, Kotz R, *et al*. Neoadjuvant chemotherapy for osteogenic sarcoma: results of a cooperative German/Austrian study. *J Clin Oncol* 1984, **2**, 617–624.
3. Winkler K, Beron G, Delling G, *et al*. Neoadjuvant chemotherapy for osteogenic sarcoma: results of a randomized cooperative trial (COSS-82) with salvage chemotherapy based on histological tumor response. *J Clin Oncol* 1988, **6**, 329–337.

4. Bacci G, Picci P, Ferrari S, *et al*. Primary chemotherapy and delayed surgery for nonmetastatic osteosarcoma of the extremity. *Cancer* 1993, **72**, 3227–3238.
5. Saeter G, Alvegard TA, Elomaa I, *et al*. Treatment of osteosarcoma of the extremities with the T-10 protocol, with emphasis on the effects of preoperative chemotherapy with single-agent high-dose methotrexate: a Scandinavian Sarcoma Group study. *J Clin Oncol* 1991, **9**, 1766–1775.
6. Kalifa C, Razafindrakoto H, Vassal G, *et al*. Chemotherapy in osteogenic sarcoma: the experience of the pediatric department of the Goustave Roussy Institute. *Cancer Treat Res* 1993, **62**, 347–349.
7. Benjamin RS, Chawla SP, Carrasco CH, *et al*. Preoperative chemotherapy for osteosarcoma with intravenous doxorubicin and intra-arterial cis-platinum. *Ann Oncol* 1992, **2**, 3–6.
8. Provisor AJ, Ettinger LJ, Nachman JB, *et al*. Treatment of nonmetastatic osteosarcoma of the extremity with preoperative and postoperative chemotherapy: a report from the Children's Cancer Group. *J Clin Oncol* 1997, **15**, 76–84.
9. Wunder JS, Paulian G, Huvos AG, *et al*. The histological response to chemotherapy as a predictor of the oncological outcome of operative treatment of Ewing sarcoma. *J Bone Joint Surg Am* 1998, **80**, 1020–1033.
10. Bielack SS, Kempf-Bielack B, Delling G, *et al*. Prognostic factors in high-grade osteosarcoma of the extremities or trunk: an analysis of 1702 patients treated on neoadjuvant Cooperative Osteosarcoma Study Group protocol. *J Clin Oncol* 1991, **9**, 1766–1775.
11. Huvos AG, Rosen G, Marcove RC, *et al*. Pathologic aspects in 20 patients after treatment with chemotherapy, en bloc resection, and prosthetic bone replacement. *Arch Pathol Lab Med* 1977, **101**, 14–18.
12. Salzer-Kuntschik M, Brand G, Delling G, *et al*. Bestimmung des morphologischen regressionsgrade nach chemotherapie bei malignen knochetumoren. *Pathologe* 1983, **4**, 135–141.
13. Ayala AG, Raymond KR, Jaffe N, *et al*. The pathologist's role in diagnosis and treatment of osteosarcoma in children: controversies, current status and projections. *Hum Pathol* 1984, **15**, 258–266.
14. Bacci G, Picci P, Ruggieri P, *et al*. Primary chemotherapy and delayed surgery (neoadjuvant chemotherapy) for osteosarcoma of the extremities. The Istituto Ortopedico Rizzoli experience in 127 patients treated preoperatively with intravenous methotrexate (high *versus* moderate doses) and intraarterial cisplatin. *Cancer* 1990, **65**, 2539–2553.
15. Goorin AM, Schwartzentruber DJ, Devidas M, *et al*. Presurgical chemotherapy compared with immediate surgery and adjuvant chemotherapy for nonmetastatic osteosarcoma: Pediatric Oncology Group Study POG-8651. *J Clin Oncol* 2003, **21**, 1574–1580.
16. Enneking WF, Spanier S, Goodman MA, *et al*. A system for the surgical staging of musculoskeletal sarcomas. *Clin Orthop* 1980, **153**, 106–120.
17. Picci P, Bacci G, Campanacci M, *et al*. Histologic evaluation of necrosis in osteosarcoma induced by chemotherapy. *Cancer* 1985, **56**, 1515–1521.
18. Bacci G, Ferrari S, Mercuri M, *et al*. Neoadjuvant chemotherapy for extremity osteosarcoma. Preliminary results of the Rizzoli's 4th Study. *Acta Oncol* 1998, **37**, 41–48.
19. Ferrari S, Mercuri M, Picci P, *et al*. Nonmetastatic osteosarcoma of the extremity: results of a neoadjuvant chemotherapy protocol (IOR/OS-3) with high-dose methotrexate, intraarterial or intravenous cisplatin, doxorubicin, and salvage chemotherapy based on histologic tumor response. *Tumori* 1999, **85**, 458–464.
20. Bacci G, Ferrari S, Longhi A, *et al*. High-dose ifosfamide in combination with high-dose methotrexate, doxorubicin and cisplatin in the neoadjuvant treatment of extremity osteosarcoma: preliminary results of an Italian Sarcoma Group/Scandinavian Sarcoma Group pilot study. *J Chemother* 2002, **14**, 198–206.
21. Bacci G, Ferrari S, Bertoni F, *et al*. Long-term outcome for patients with nonmetastatic osteosarcoma of the extremity treated at Istituto Ortopedico Rizzoli accordino to the IOR/OS-2 protocol: an updated report. *J Clin Oncol* 2000, **18**, 4016–4027.